Comparative Study of Spontaneous and FUdR Induced Chromosomal Instability in Pakistani Lohi and British Suffolk Sheep – An Indication of Environmental Mutagenesis

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Abstract.- A Study to compare the extent of geno-environmental interaction expressed as fragile sites was carried out in British Suffolk and Pakistani Lohi breeds of sheep (Ovis aries). Spontaneous and 5-Fluorodeoxyuridine (FUdR) induced fragile sites were scored in metaphase chromosomes in two flocks of sheep reared under different environmental conditions. Means and standard errors for aberrant cell count (AC) and number of aberrations (NoA) per animal revealed highly significant differences between the two groups (P<0.01) belonging to distant continents of the globe. Average Number of cells with gaps and breaks (AC) was 0.56±0.15 Vs 1.91±0.34 whereas NoA index per cell averaged 0.59±0.16 Vs 2.36±0.48 in chromosomes grown in control cultures of Lohi and Suffolk breeds respectively. Corresponding means in FUdR synchronized chromosomes were 2.18±0.33 Vs 13.26±0.85 and 2.65±0.50 Vs 21.87±1.88 in the two breeds respectively. Distribution comparison of chromosomal fragile sites grown in control cultures with no FUdR revealed seven fragile bands in Lohi compared to 24 in Suffolk. Microscopic analysis for the number of fragile bands in FUdR treated chromosomes revealed 29 and 78 autosomal bands in Lohi and Suffolk breeds respectively. Similarly the number of significantly fragile bands was 4 and 78 in the two breeds respectively. X-chromosome in Lohi was highly stable in control as well as FUdR cultures expressing no gaps and breaks whereas the Suffolk X expressed 4 fragile bands. The study suggested that long term exposure of the two flocks to entirely variable environment might have played a significant role for higher chromosomal damage in the Suffolk breed.

Keywords: Spontaneous chromosomal instability, FUdR, chromosomal instability, Pakistani Lohi, British Suffolk, environmental mutagenesis.

INTRODUCTION

With new molecular cloning insights regarding fragile sites of clinical and common importance the interest in the subject has been renewed. Although fragile sites in human have been the major point of interest with only a small number of studies cited in farm animals (Riggs and Ronne, 2009). Similarily Ali et al. (2008) published a detailed overview fragile site phenomenon in sheep genome.

Although the exact mechanism of fragile site expression still remains unclear, however some external factors have been implicated to be instrumental for their expression.

Environmental pollution is an important factor for increased rates of mutagenesis due to higher than ever industrial and automobile emissions (Darkwa, 1996). A number of reports are available describing spontaneous and chemically induced chromosomal damage in living organisms due to their exposure to heavy pollutants in the environment. Higher chromosomal lesions, congenital abnormalities and higher rates of dynamic mutations are caused by long term exposure of animals and plants to air, water and land born pollutants (Ahmad et al., 1998; Carluse et al., 2005; Di Berardino et al., 1983). A unique comparative investigation was planned to explore the extent of environmental genotoxicity expressed as fragile sites, in Lohi breed of Pakistan exposed to relatively pure and rural environment in the remote area of District, Okara with minimal automobile and industrial emissions compared to Suffolk sheep exposed to a highly industrial and polluted environment of Avonmouth area in Bristol,

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Fragile sites are chromosomal regions frequently showing gaps, breaks and multi-radial figures in-vitro spontaneously or when treated with some carcinogenic substance such as FUdR Carlose et al. (2005). This investigation was carried out to compare the extent of spontaneous and FUdR induced fragile sites in Pakistani Lohi and British Suffolk sheep.

**MATERIALS AND METHODS**

Cytogenetic data on two measures of chromosomal damage *viz.* aberrant cell count (AC), defined as the number of metaphase cells showing gaps and breaks in chromosomes or chromatids and the number of aberrations, (NoA) defined as total number of gaps and breaks in a single metaphase, was collected from 45 Suffolk and 32 Lohi sheep breeds. Blood samples of Suffolk sheep were obtained in heparinized vacutainers through jugular venepuncture and cultured in Cytogenetic Unit of the Department of Clinical Veterinary Science, Langford University of Bristol UK. Same procedure was followed to collect peripheral blood samples from 20 healthy and adult ewes and 12 breeding rams from a flock of Lohi breed maintained at Livestock Production Research Institute Bahadarnagar Okara, Pakistan and processed at the Centre of Excellence in Molecular Biology (CEMB), Lahore, Pakistan. Lohi animals were confined to pollution free environment for generations at the farm area covering about 2000 acres of land in the heart of a rural area and largely surrounded by agri-based industry.

![Fig. 1. Spontaneous and FUdR induced fragile site map in British Suffolk cross sheep.](image1)

![Fig. 2. Fragile site in Lohi sheep.](image2)
Table I.- Comparison of the expression of folate sensitive chromosomal lesions between British and Pakistani sheep breeds at different FUdR levels.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>5µg/ml</th>
<th>5µg/ml</th>
<th>Test statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lohi</td>
<td>Suffolk</td>
<td>Lohi</td>
</tr>
<tr>
<td>Average AC per animal</td>
<td>0.56±0.15</td>
<td>1.91±0.34</td>
<td>2.18±0.33</td>
</tr>
<tr>
<td>Average NoA per animal</td>
<td>0.59±0.16</td>
<td>2.36±0.48</td>
<td>2.65±0.50</td>
</tr>
</tbody>
</table>

F0530) 24 hour prior to harvesting. Blood cultures were harvested using standard harvesting technique. The slides were prepared and giemsa stained for microscopy.

Each slide prepared from control as well as experimental cultures, was screened to record cytogenetic data on AC and NoA. A light microscope (oil immersion x1000) was used to screen 50 good quality mitotic spreads in control and FUdR treated cultures from each animal. Aberrant cells were photographed with a manual orthomate camera using Kodak Technical Pan film. The slides were then de-stained with 3:1 methanol acetic acid fixative and stored at –20 °C until ready for GTG-banding using standard technique (Seabright, 1971). Aberrant cells with optimal GTG-bands were re-photographed and chromosomal lesions were mapped to individual chromosomal bands using G-band nomenclature (Di Berardino et al., 1983). The data on AC and NoA was analysed using Mann-Whitney, a non-parametric statistical test (Minitab Statistical Software Release 3.11) to determine variation in the frequency of fragile site expression between different treatments and groups. Individual chromosomal band data with gaps and breaks was analysed to identify statistically fragile bands (fragile sites) using multinomial statistical model (Bohm et al., 1995).

RESULTS

A comparison of AC and NoA means with standard errors between British Suffolk and Pakistani Lohi is summarised in Table I. The AC index from control cultures was 1.12% and 3.70% in Lohi and Suffolk breeds respectively. AC means per animal in metaphase cells grown in the control cultures were 0.56±0.15 and 1.91±0.34 in two breeds respectively. Statistical comparison revealed variation in AC means to be significant (P<0.05) between the two groups with Suffolk sheep expressing approximately three times more chromosomal breaks and gaps and breaks compared to Lohi animals. Chromosomal instability in Suffolk breed was manifold in 5 µg/ml FUdR treated chromosomes. Consequently AC index was 4.34% with corresponding average values per animal being 2.18±0.33 respectively in Lohi and Suffolk breeds. The scale of variation for AC means in control cultures of Suffolk was high enough to be comparable with FUdR treated cultures from Lohi breed.

NoA was taken as a measure of the extent of chromosomal instability within each cell of an individual animal to reflect the scale of chromosome damage in spontaneously or induced cells in overall metaphase cells scored and recorded. Individual NoA means in metaphase cells grown in control cultures was 1.91 ± 0.34 in Suffolk sheep. Statistical comparison revealed variation in NoA means to be significant higher (P<0.05) when compared between the two groups. Genomic instability following 5µg/ml FUdR treatment in Suffolk breed was also recoreded to be exceptionally higher for breed comparison exposed to different environmental condition. Calculated NoA index was 28.33% with corresponding average NoA values per animal being rising from 2.18 ± 0.33 in Lohi sheep to 13.26 ± 0.85 respectively in Suffolk breed. The scale of variation for AC and NoA means in control cultures of Suffolk was high enough to be comparable with FUdR treated cultures from Lohi breed.

Distribution comparison between Lohi and Suffolk breeds revealed significant variation in the number of individual chromosomal bands expressing gaps and breaks (Table II). It was clearly
obvious that fewer chromosomal lesions were expressed in fewer chromosomal bands in Lohi genome expressing only 7 fragile bands compared to 24 bands in the Suffolk breed in control (spontaneous chromosome damage) cultures. However, FUdR treated chromosomes in the two breeds revealed 29 and 78 autosomal bands respectively. Similarly the number of statistically fragile bands in Lohi genome was 4 compared to 58 in the Suffolk breed. Lohi X-chromosome also demonstrated high level of stability even in FUdR treated cultures with no fragile sites identified.

**DISCUSSION**

Number of reports describing significant variation in the expression of folate sensitive fragile sites from different subjects depending upon age, health status, breed and sex in different species are available (Carlose et al., 2005; Di Berardino et al., 1983; Kahkönen, 1988). Some evidence also exists on non significant breed variation in the expression of folate sensitive fragile sites between two breeds of dog (Stone et al., 1991) however, no studies have reported variation of the magnitude found in the present study.

There are a number of reports describing significantly higher chromosomal damage in mammals exposed to high level of organic environmental pollutants (Ahmad et al., 1998; Carlose et al., 2005; Stone et al., 1991; Hongell, 1996; Iredi et al., 1998; Randerath, 1999; Roderiguez et al., 1997). High organic energy usage by industry and automobiles, in turn, significantly increases the levels of environmental pollutants. It has been described that energy consumption in various sectors in the United Kingdom is associated with high emissions of gasses such as CO₂, SO₂, oxides of nitrogen (NOx), CO and volatile organic compounds (VOC). United Kingdom alone is reported to produce 3% of the world’s total emission of CO₂ per year (Darkwa, 1996). In addition to the global climate change, the biological impact of environmental pollutants sets a foundation to accelerate environmental mutagenesis. This leads to micro and macro level mutations in chromosomal DNA resulting in elevated incidence of congenital abnormalities, developmental malformations,
aborted and fertility problems (Iannuzzi and Di-Berardino, 2008). About 25% of abortions and 2.5% of abnormal foetuses in two flocks of sheep raised in Naples were reported to be a direct result of high level of dioxins in the environment (Schoket, 1999). Similarly, significantly higher chromosomal lesions, congenital abnormalities and higher rates of dynamic mutations both in animals and humans have been reported (Sharara et al., 1998; Sriram et al., 1999; Uchida et al., 1986; Yang and Long, 1993). It may be concluded from the chromosomal instability data presented in this study that the genomic stability exhibited by Lohi genome is attributable to its exposure to environment with relatively low level of industrial and automobile pollutants compared to Suffolk which is raised in a heavily industrial area in UK.

The analysis of spontaneously occurring and induced fragile sites also contributes to better understanding the processes shaping mammalian genome evolution. Common fragile sites are usually termed as the hotspots in the mammalian genome which tend to breakaway and relocate on different other points of the same significance to evolve new combinations of sometimes positive nature (evolution) or negative nature in the form of deleterious mutations (Sutherland and Hecht, 1985). Correlation of translocation breakpoints with fragile sites in the present study (data not presented) and elsewhere suggests high coincidence of break sites in the two phenomena (Yang and Long, 1993; Ronne, 1997). Some scientific evidence also exists comparing the points of common fragile sites with breakpoints of evolutionary significance in different species. A high level of coincidence was found between two types of breakpoints, especially in the case of X-chromosome due to its wide spread conservation among different mammalian species Smeets and Klundert (1990); Stone et al. (1991); Ronne (1992).

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